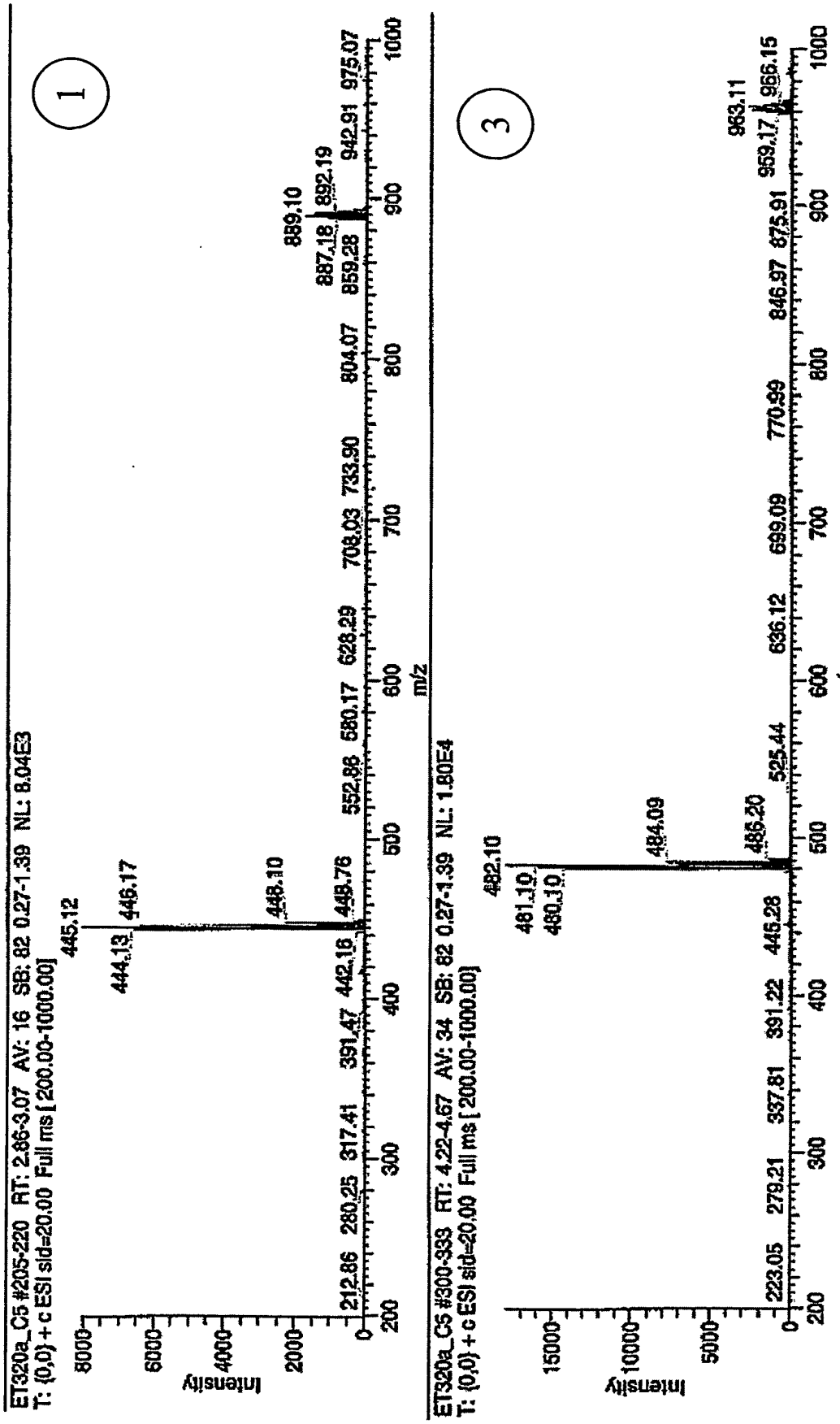


Fig. 1: Observed mass spectra ET320a1 & ET320a3

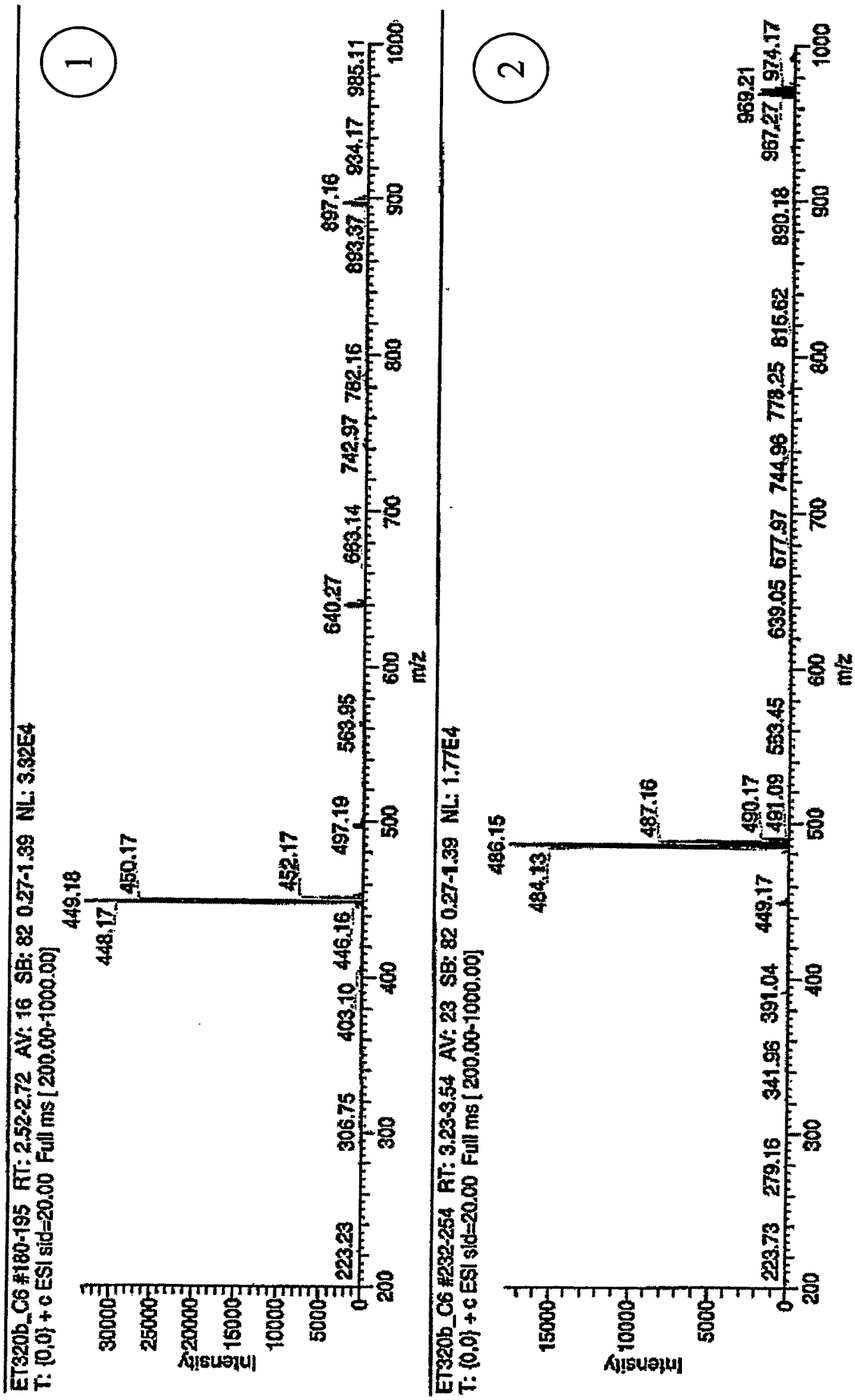


Fig. 2: Observed mass spectra ET320b1 & ET320b2

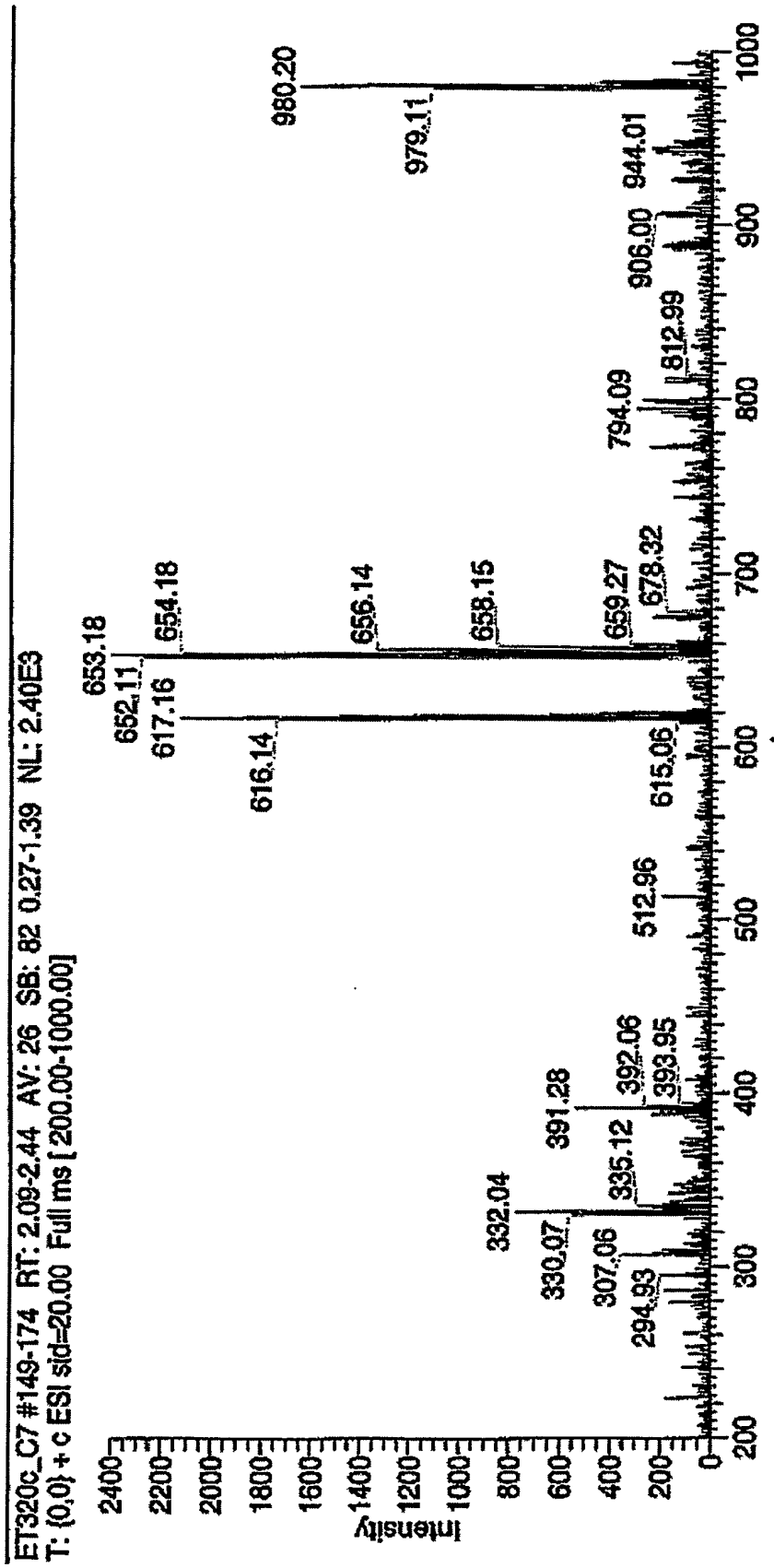


Fig. 3: Observed mass spectrum ET320c

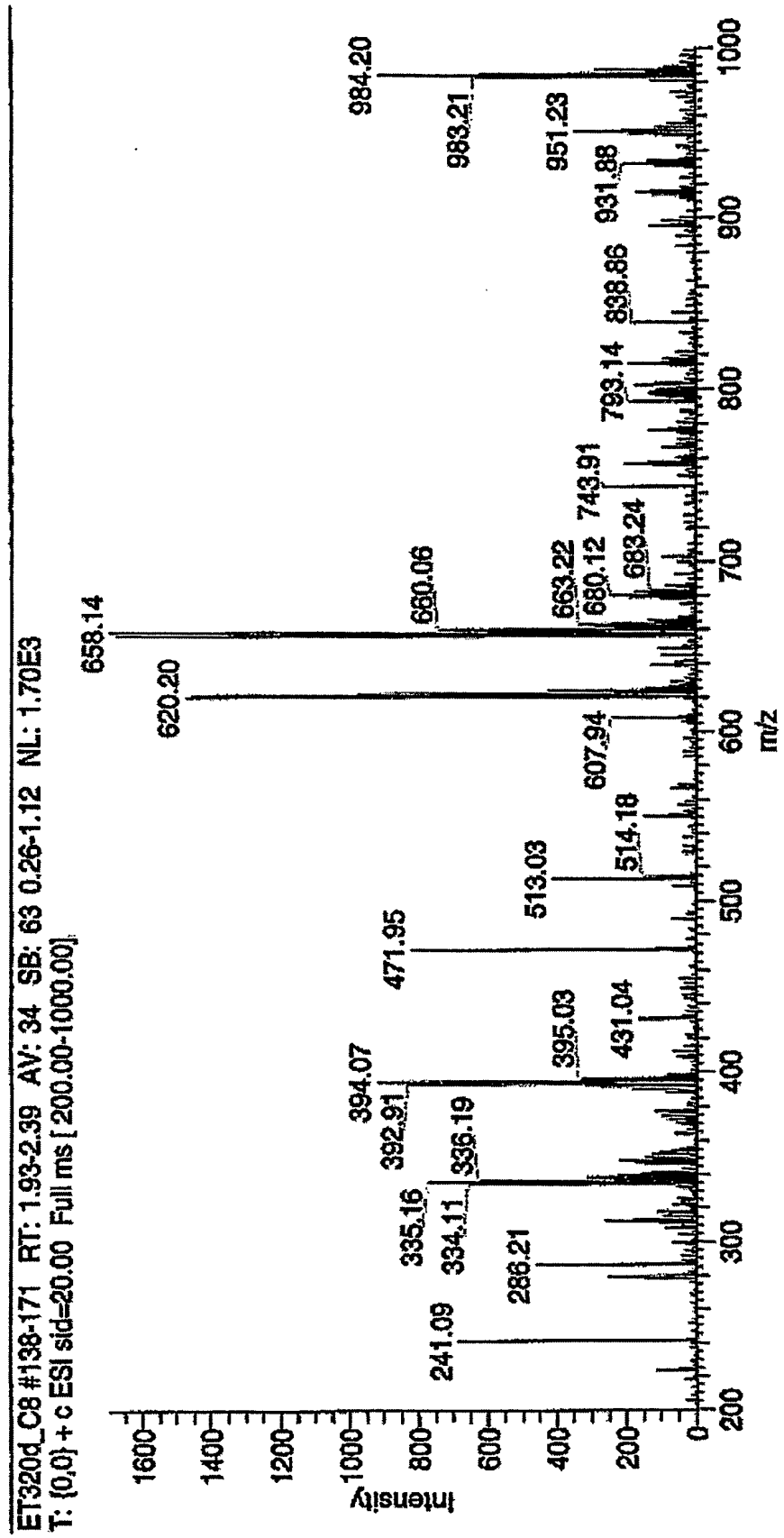
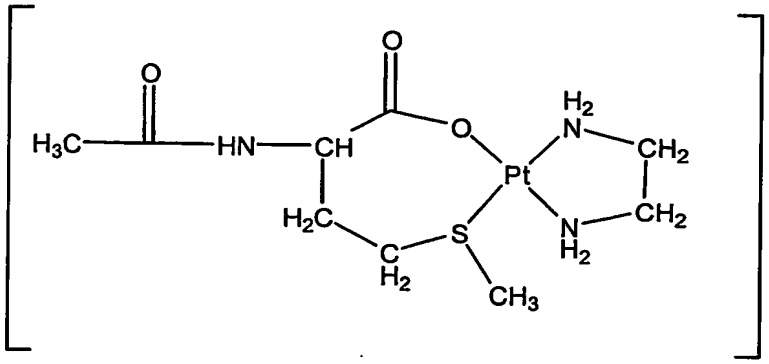


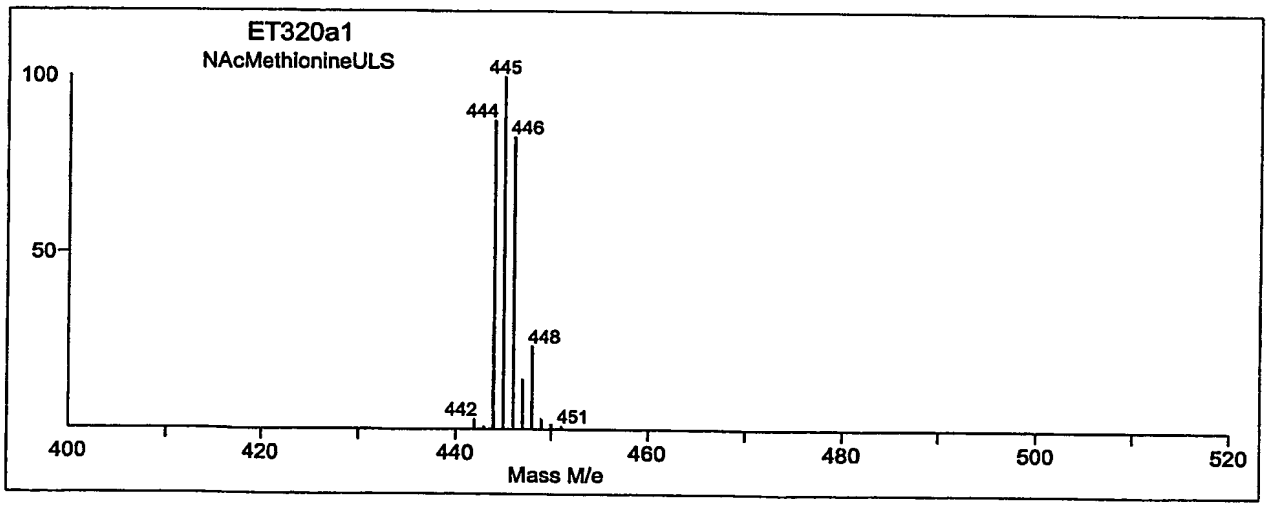
Fig. 4: Observed mass spectra ET320d

Fig. 5

ET320a1



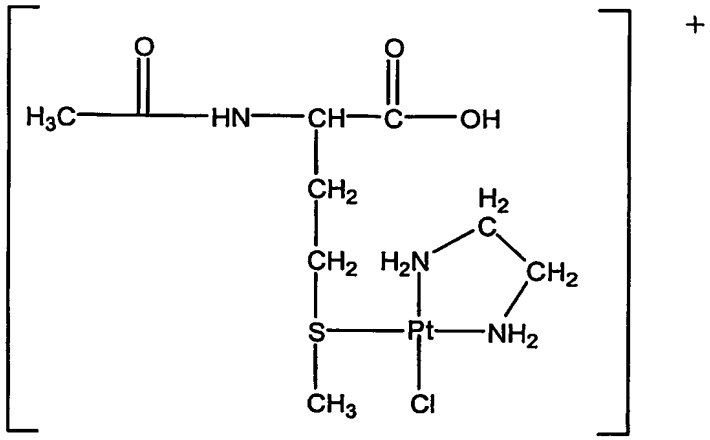
$C_9H_{20}N_3O_3PtS$
Exact Mass: 445.09
Mol. Wt.: 445.42
C, 24.27; H, 4.53; N, 9.43; O, 10.78; Pt, 43.80; S, 7.20



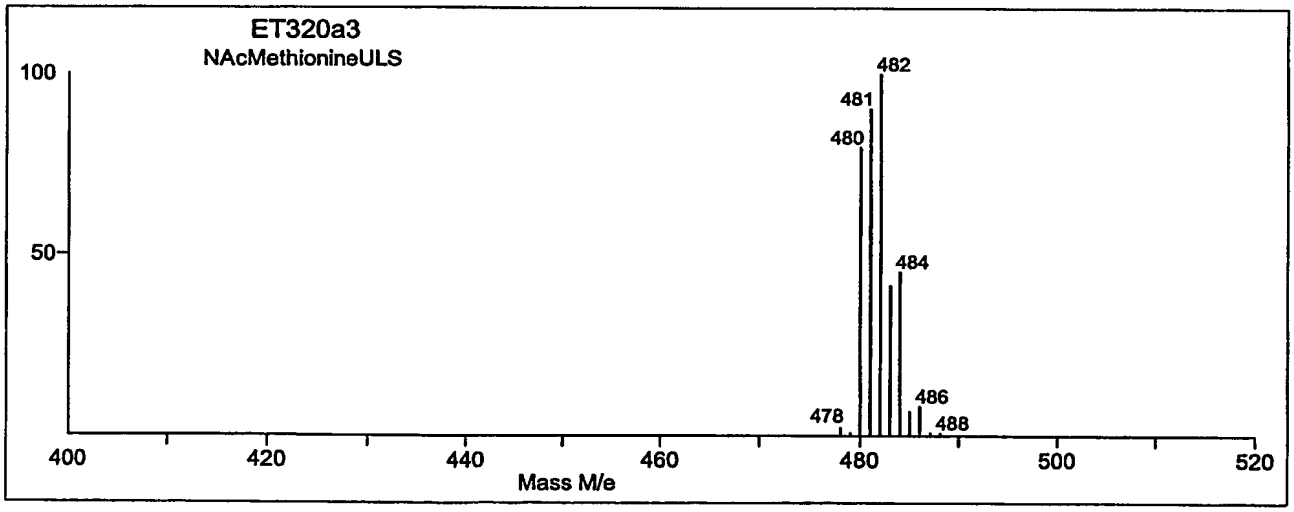
structure and theoretical mass spectrum of ET320a1

Fig. 6

ET320a3



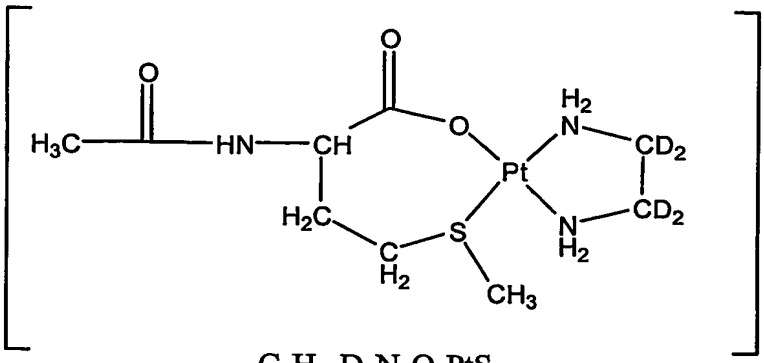
$C_9H_{21}ClN_3O_3PtS$
Exact Mass: 481.06
Mol. Wt.: 481.88
C, 22.43; H, 4.39; Cl, 7.36; N, 8.72; O, 9.96; Pt, 40.48; S, 6.65



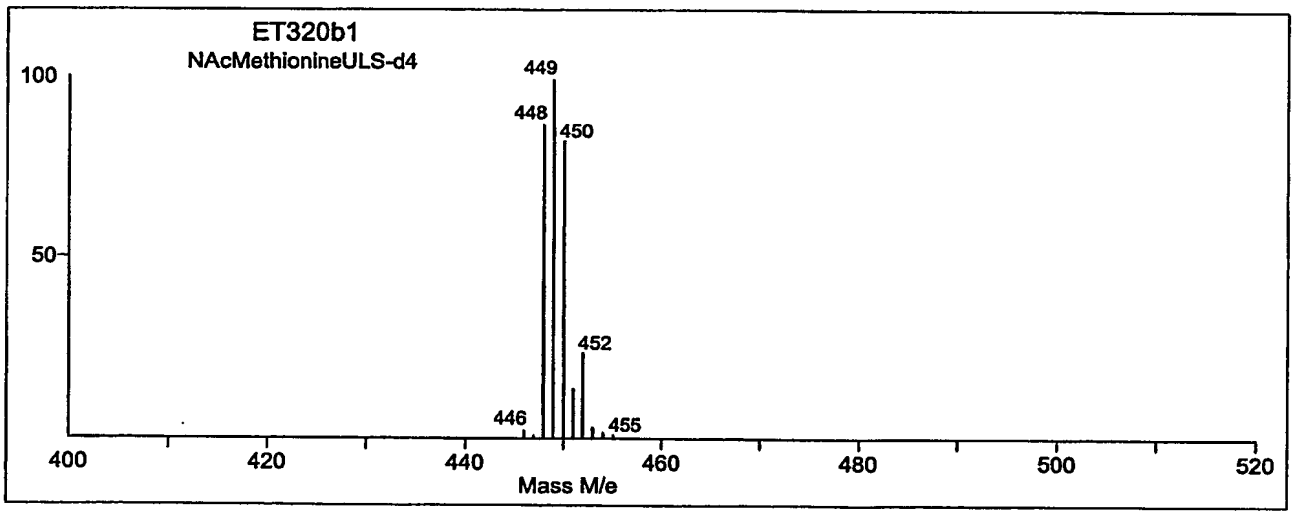
structure and theoretical mass spectrum of ET320a3

Fig. 7

ET320b1



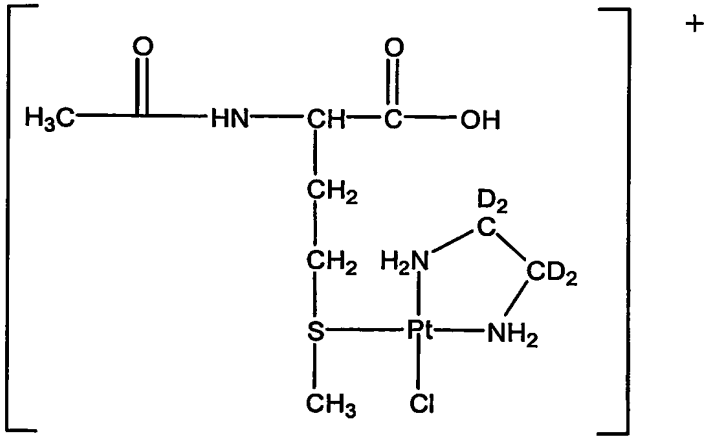
$C_9H_{16}D_4N_3O_3PtS$
Exact Mass: 449.11
Mol. Wt.: 449.44
C, 24.05; H, 5.38; N, 9.35; O, 10.68; Pt, 43.40; S, 7.13



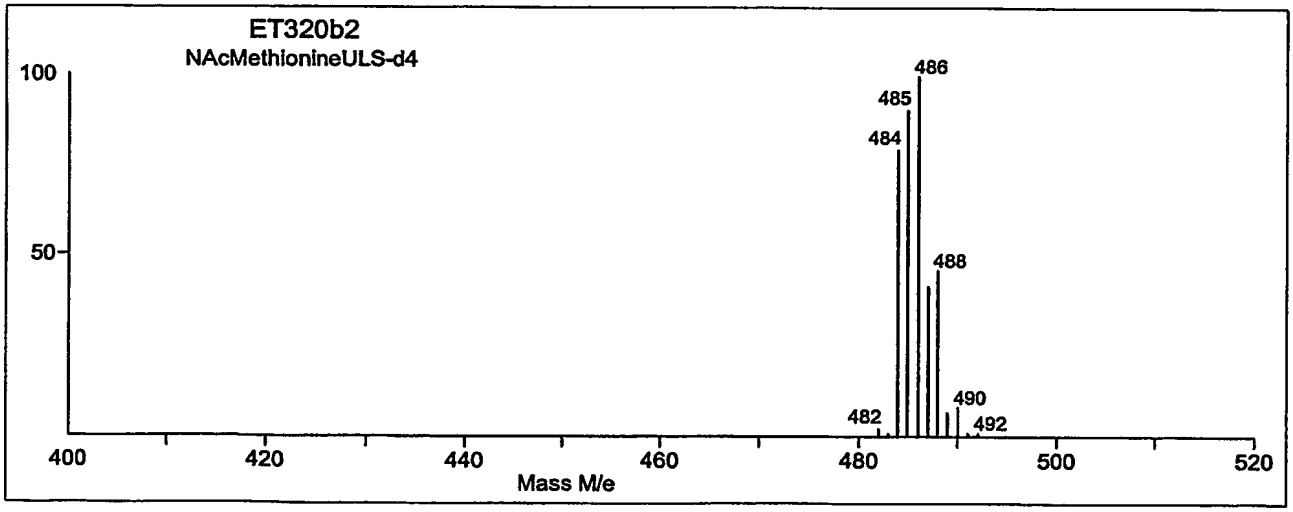
structure and theoretical mass spectrum of ET320b1

Fig. 8

ET320b2



$C_9H_{17}D_4ClN_3O_3PtS$
Exact Mass: 485.09
Mol. Wt.: 485.90
C, 22.25; H, 5.18; Cl, 7.30; N, 8.65; O, 9.88; Pt, 40.15; S, 6.60



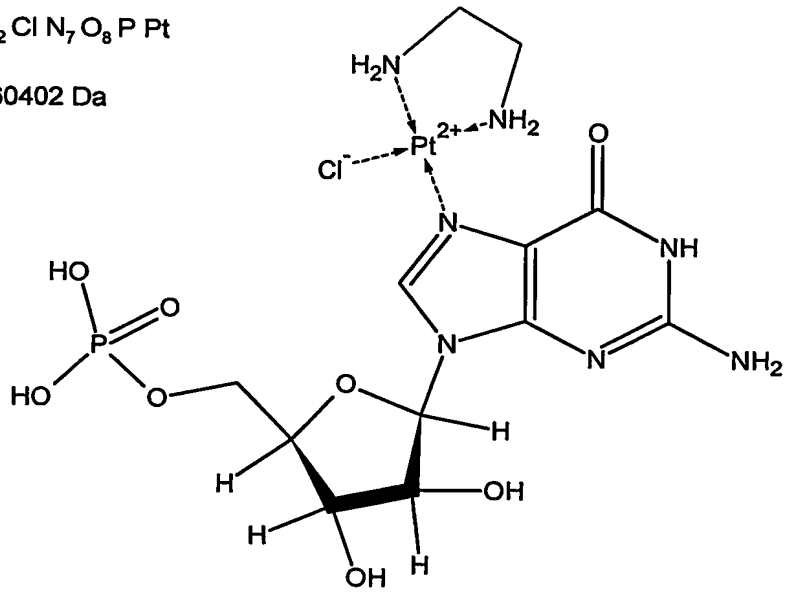
structure and theoretical mass spectrum of ET320b2

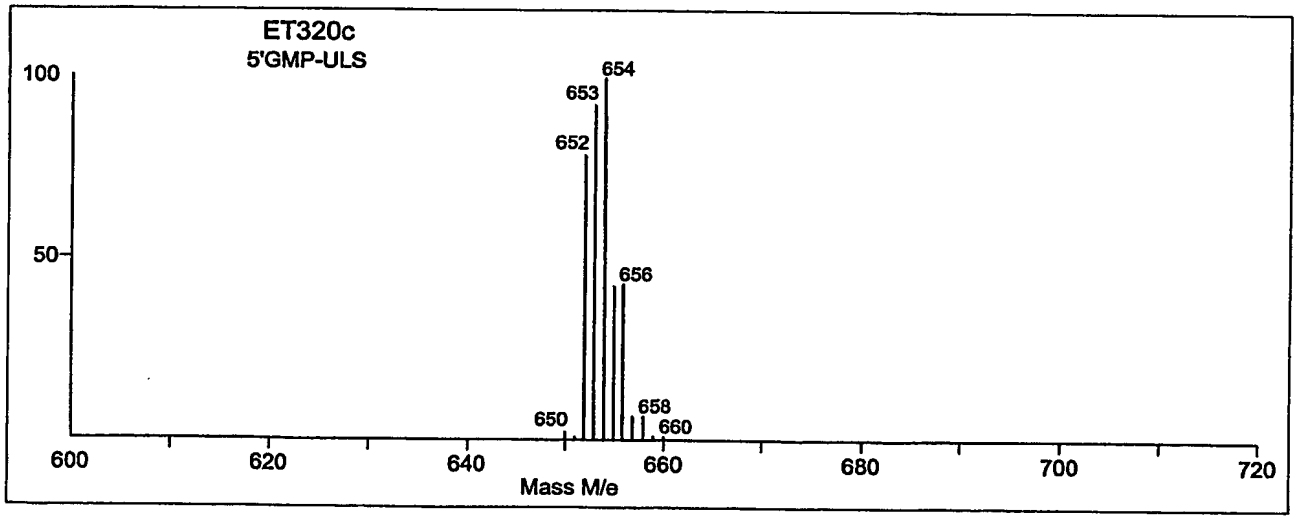
Fig. 9

ET320c

Molecular Formula = $C_{12}H_{22}ClN_7O_8PPt$

Monoisotopic Mass = 653.060402 Da





structure and theoretical mass spectrum of ET320c

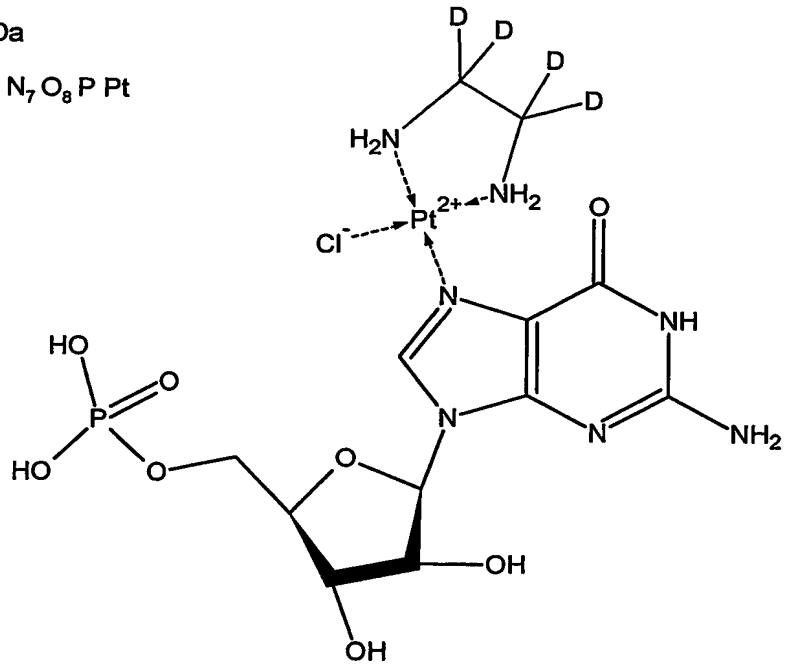
Fig. 10

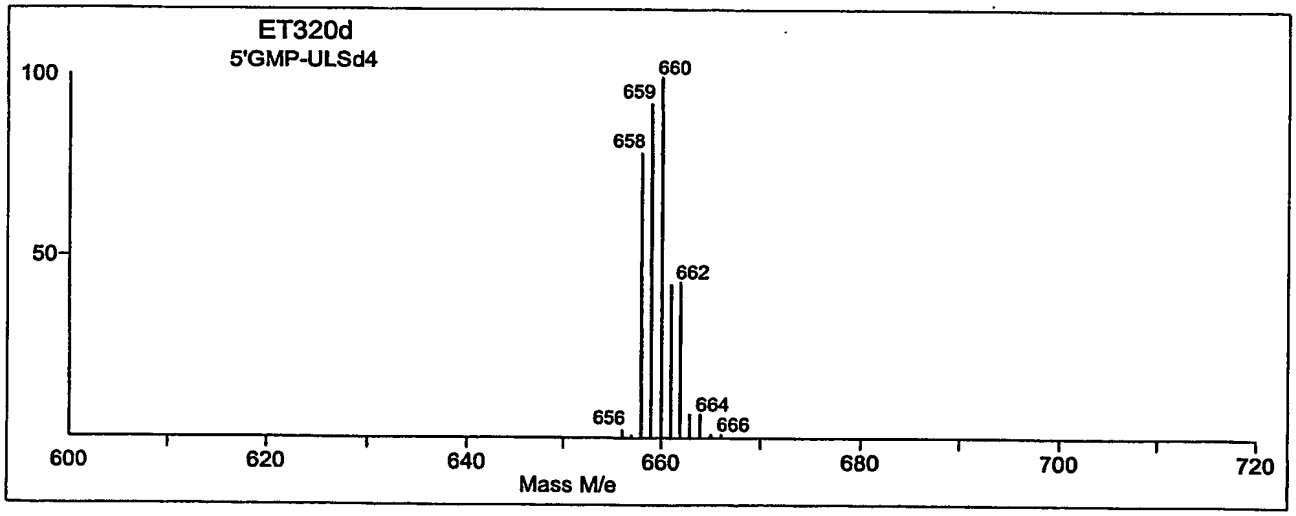
ET320d

Monoisotopic Mass = 657.08551 Da

Molecular Formula = $C_{12}H_{18}D_4ClN_7O_8PPt$

Formula Weight = 657.858

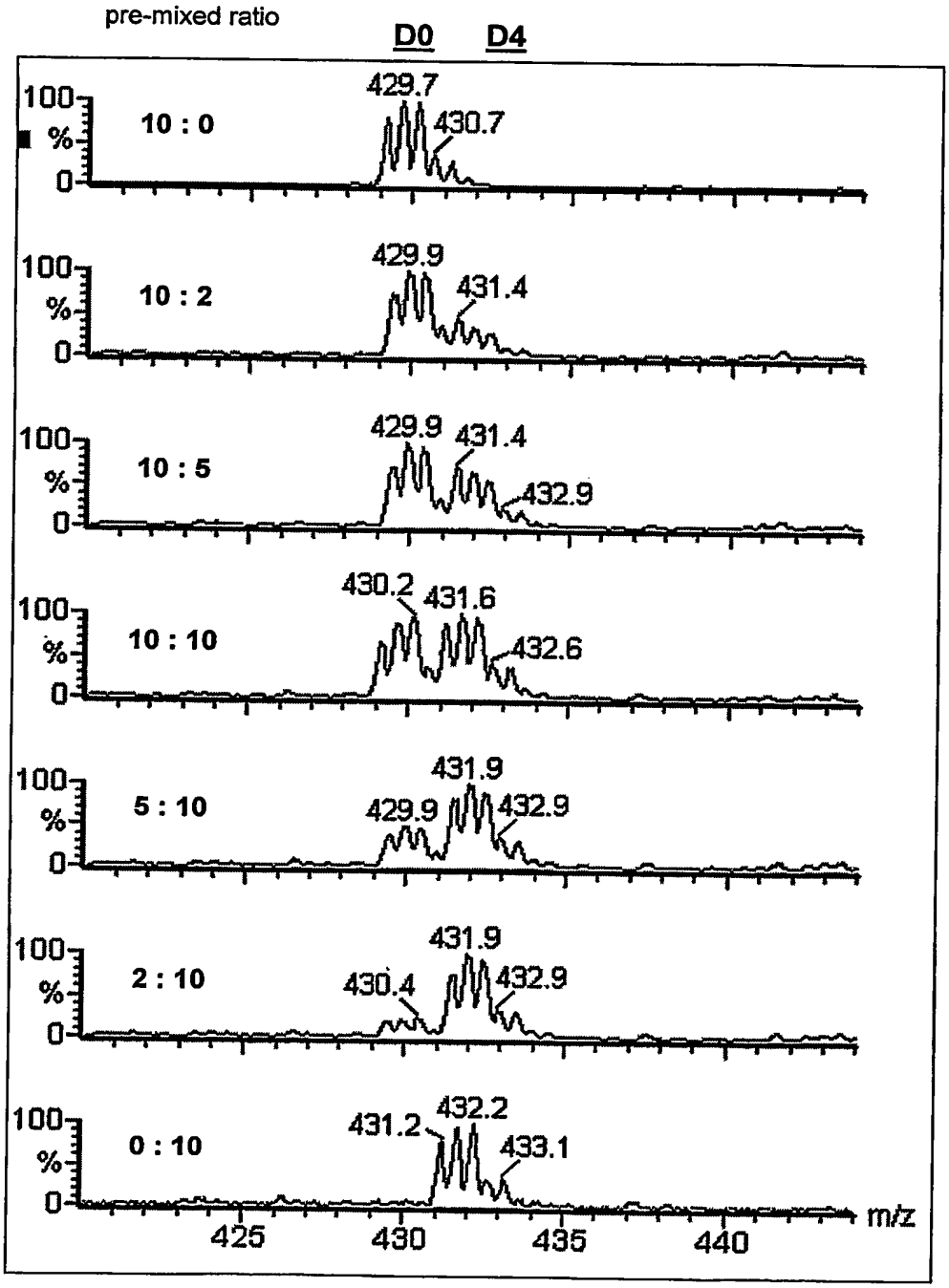




structure and theoretical mass spectrum of ET320d

Fig. 11

mass spectrometry analysis of differentially labeled LWMR peptide with light (bifunctional ULS-d0) and heavy (bifunctional ULS-d4) platinum complexes.



Note that the exact M/Z values of peaks from different spectra do not line up exactly. These minor differences are due to the fact that in some measurements (the 10:0, 10:10 and 0:10 mixtures), nanospray was used for sample application, rather than flow injection.

Fig. 12

mass spectrometry analysis of differentially labeled LWMR peptide with light (monofunctional ULS-d0-BOC) and heavy (monofunctional ULS-d4-BOC) platinum complexes.

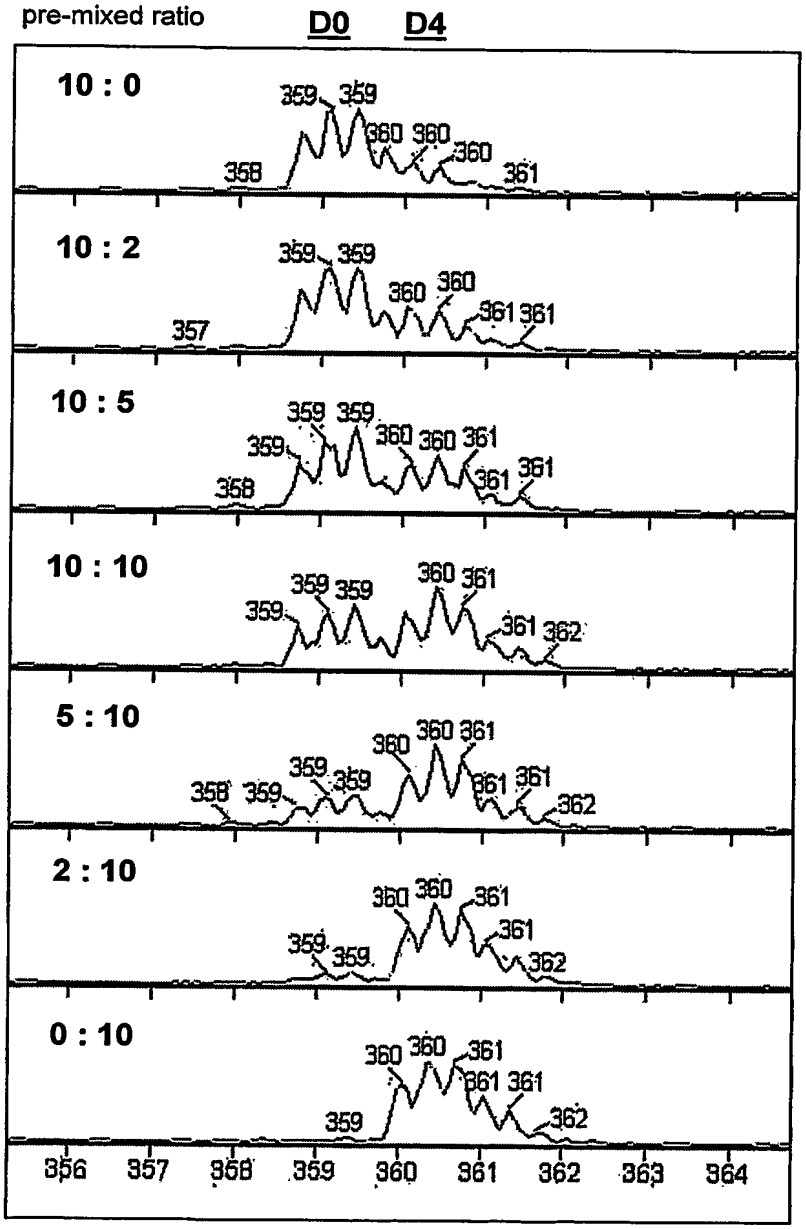
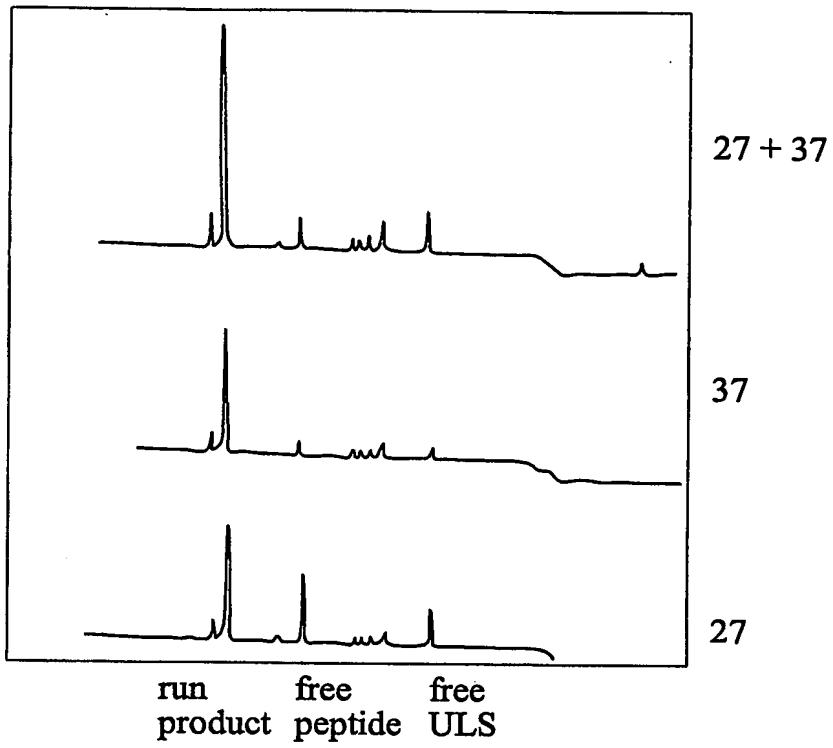
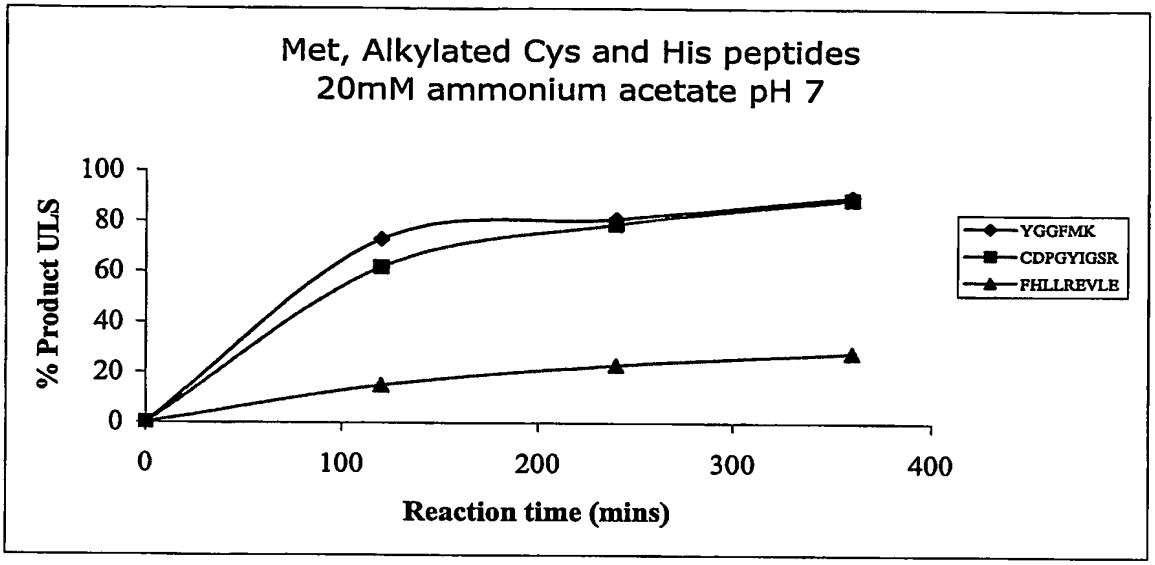


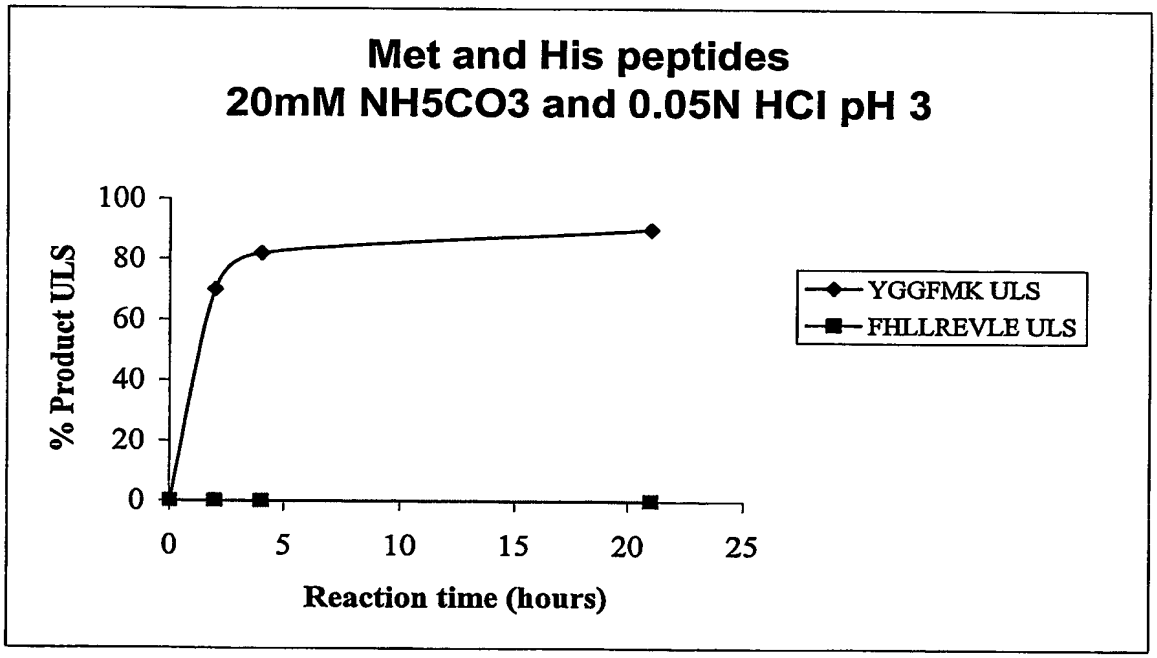
Fig. 13



HPLC of LWMR peptide labeled with monofunctional ULS-dO- BOC (37) and monofunctional ULS-d4- BOC (27) and as a mixture. Absorbance was at 220nm on a C18 (4.6mm) 5u column.

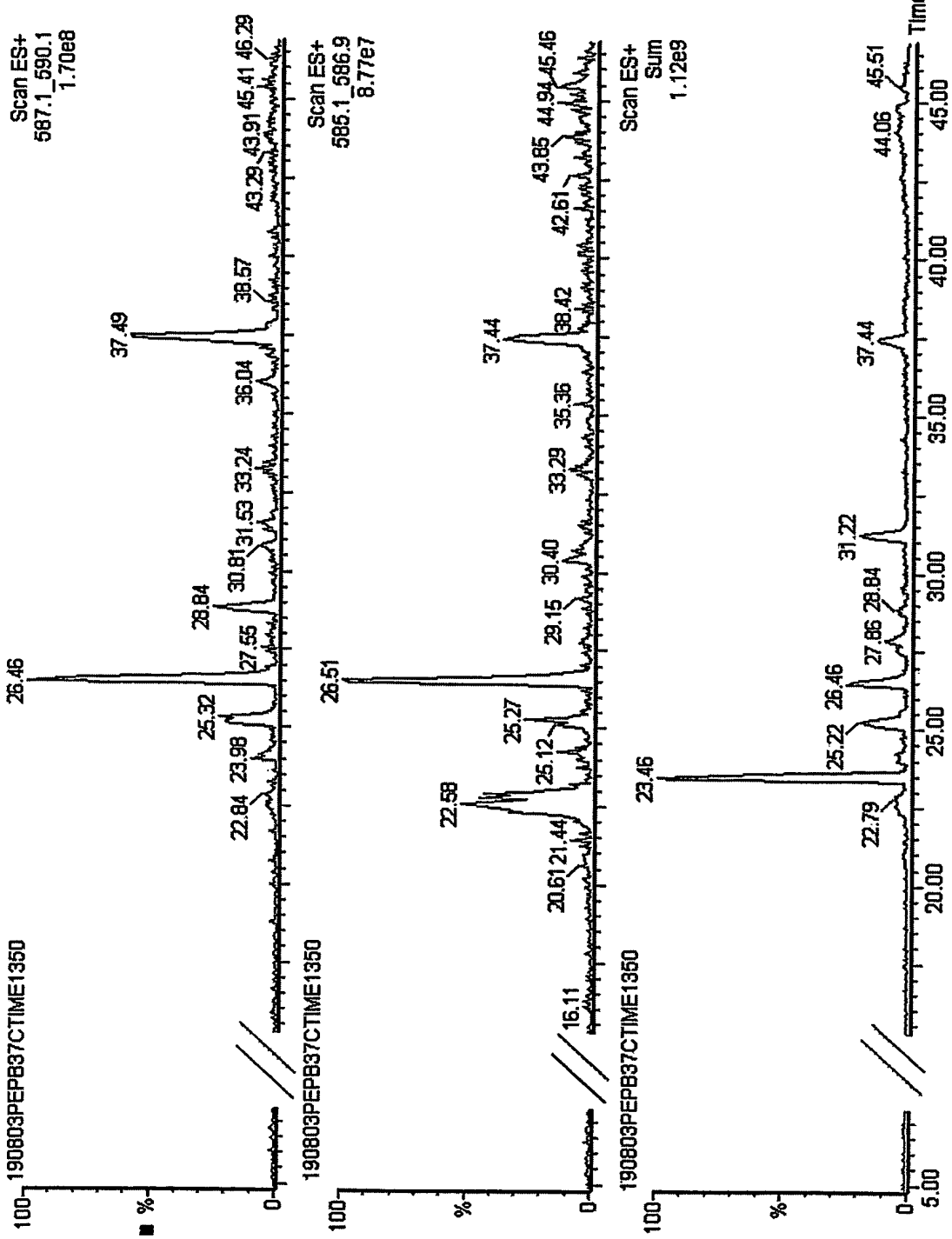
Fig. 14





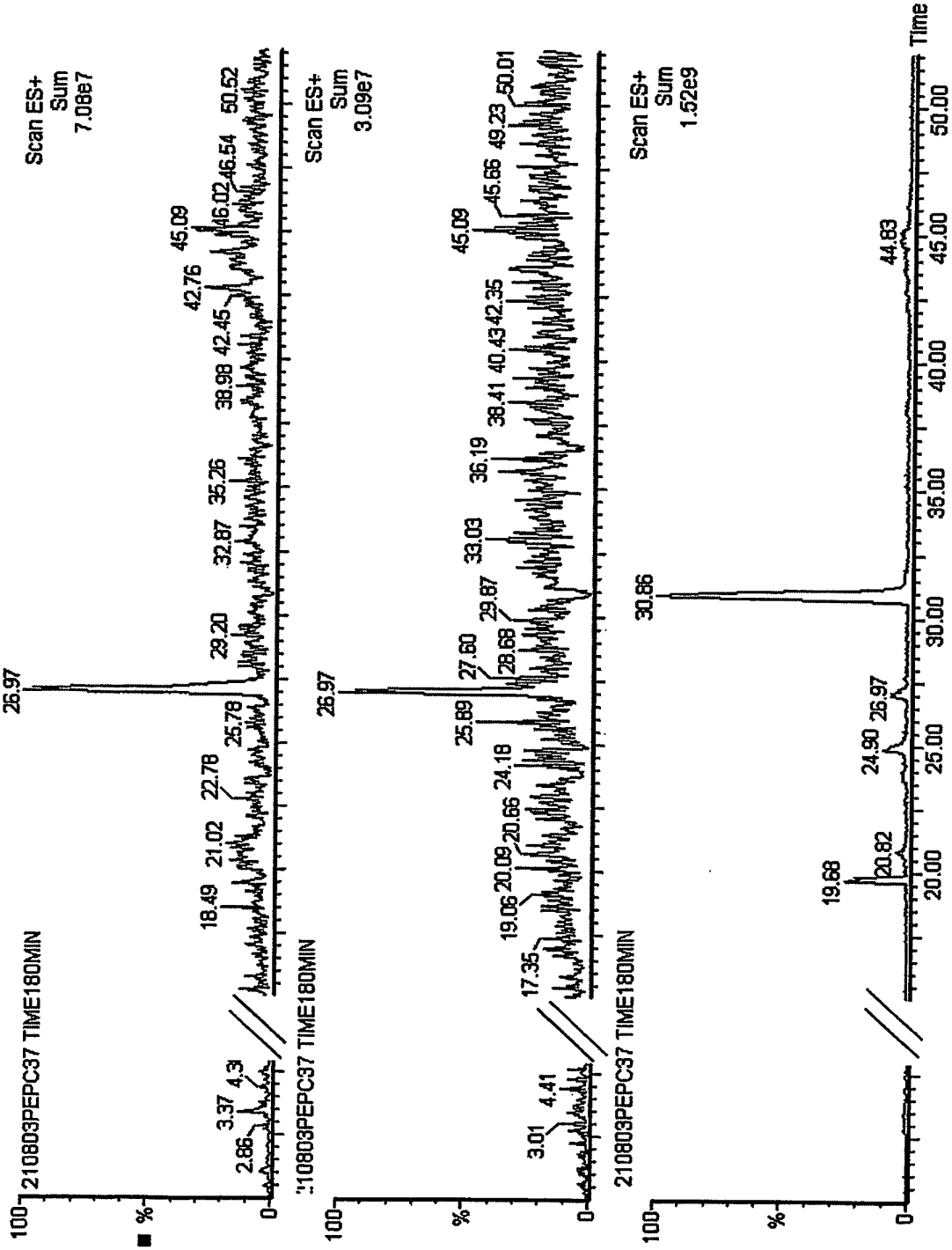
Labeling efficienty of ULS at different pH.
Labeling of peptides containing ULS targets M, C, or H with 3-fold molar excess of ULS at 45°C at pH 7(Ammonium acetate) or at pH 3 (HCl and Ammonium carbonate). Peptide YGGFM and peptide CDPGYIGSR (Cysteine iodoacetamide alkylated) and FHLLREVLE were subjected to labeling and in a time course samples were taken and subjected to ESI-MS analysis. Kinetics of labelling were derived from MS data.
A1: At pH 7 Peptide YGGFM and peptide CDPGYIGSR were labeled to saturation within 6 hrs and FHLLREVLE was labeled to 25% saturation.
A2: at pH 3 Peptide YGGFM was labeled to saturation within 8 hrs whereas peptide FHLLREVLE was not labeled at all within 21 hrs, thus low pH prevents His labelling.

Fig. 15



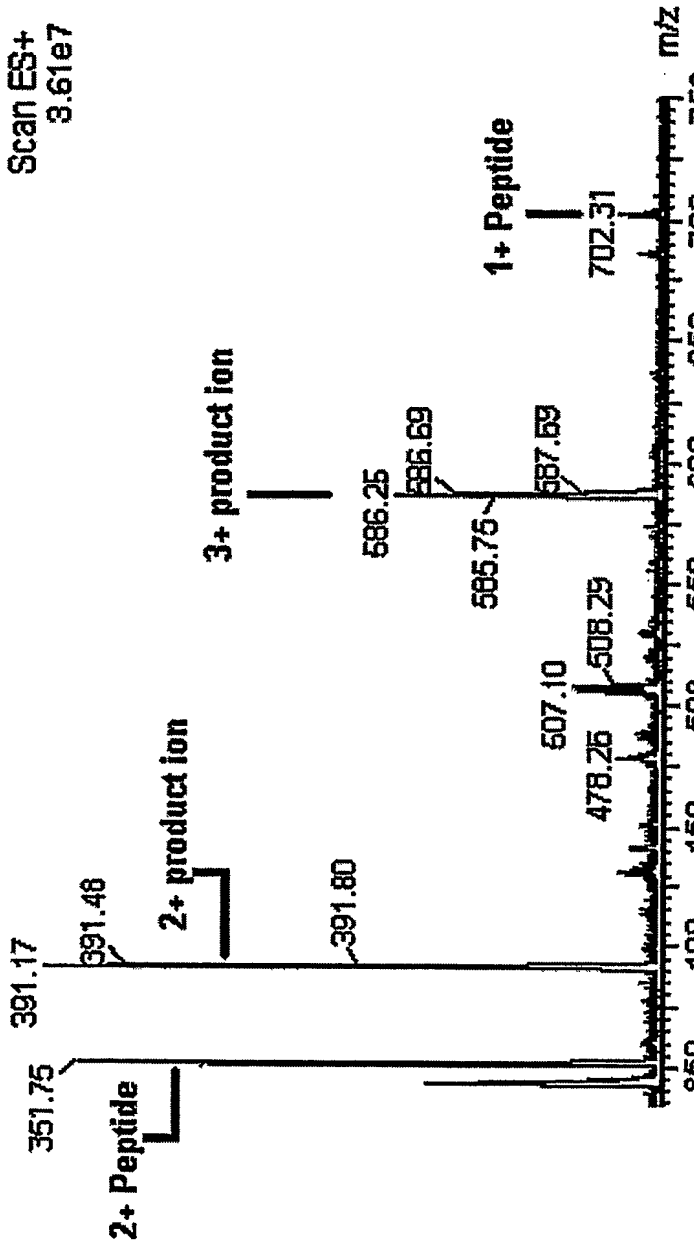
RP-LC MS TIC chromatogram of YGGFMK peptide after labeling for 22.5hrs. The lower frame shows the TIC when the free peptide (23.46 minutes), labeled peptide (26.46 minutes) and free D0 and D4 ULS are selected (37.44mins). The centre frame shows the TIC when the D0 ULS derivatised YGGFMK is selected (26.51 minutes) and the upper frame when the D4 derivitised peptide is selected (26.46 minutes).

Fig. 16



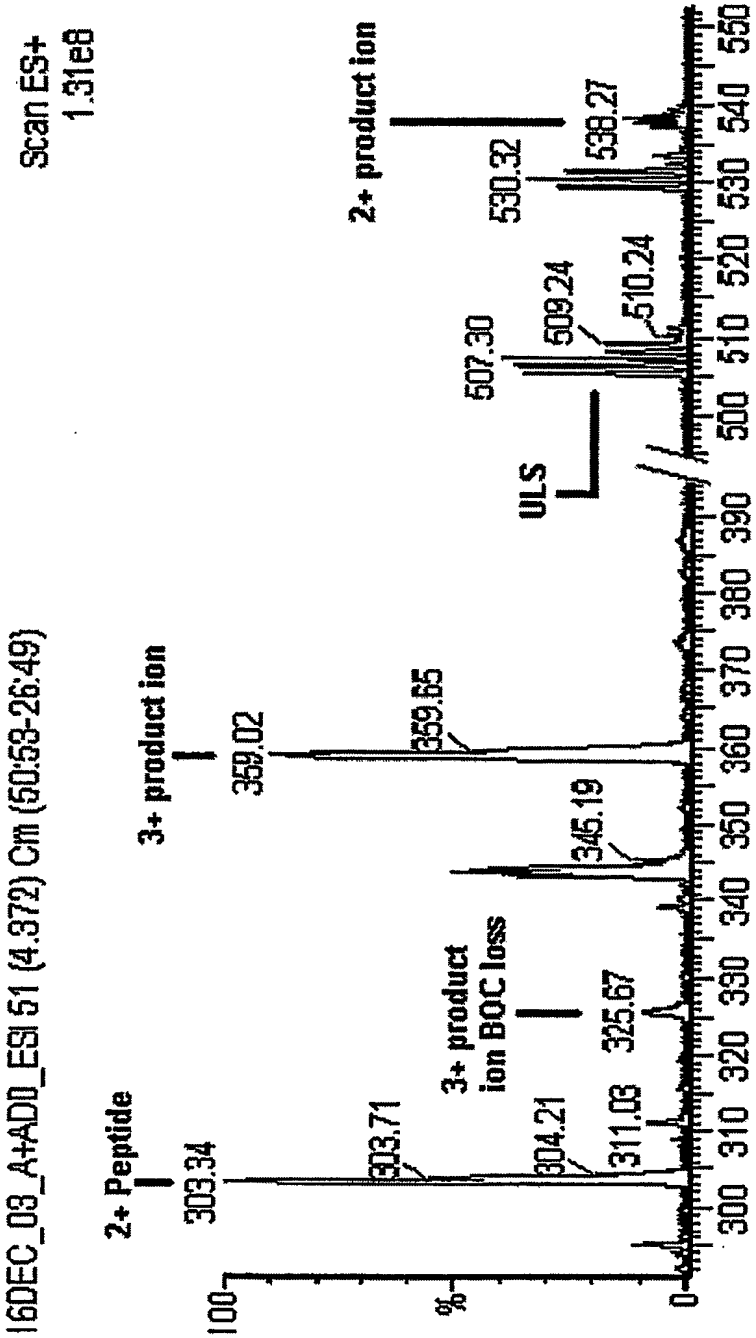
RP-LC Total Ion Chromatogram of CDPGYIGSR peptide following incubation after 2 hours with D0 and D4 ULS. The lower frame shows the TIC for the free peptide with a retention time of 19.68 minutes, labeled peptide at 26.97 minutes and free label D0 and D4 ULS at 30.86 minutes. The centre frame shows the retention time of the D0 derivitised peptide (26.97 minutes), the top frame shows the retention time of the D4 derivitised peptide (26.97 minutes).

Fig. 17



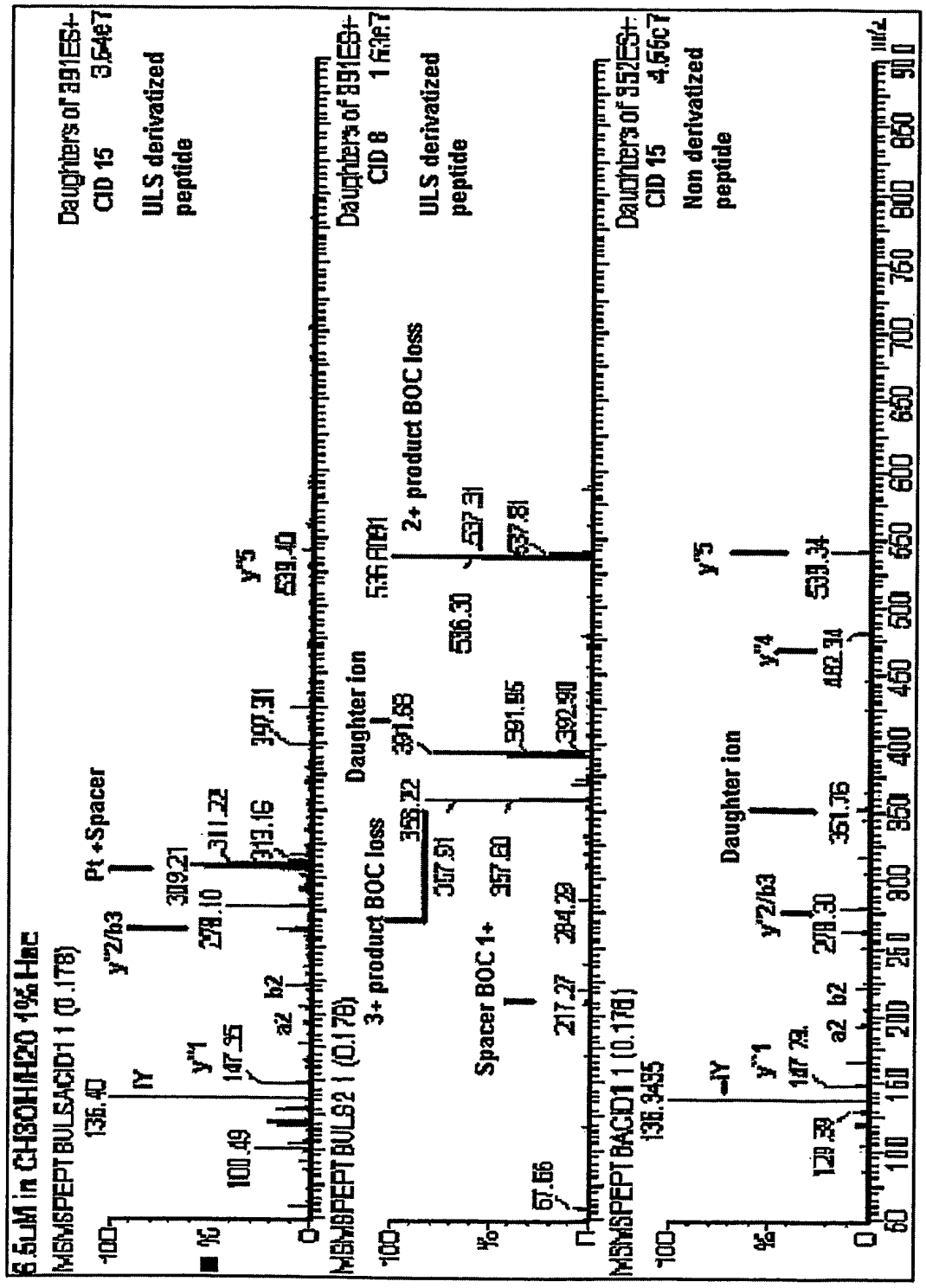
Nanospray ESI of YGGFMK peptide, showing ionisation efficiency of free peptide and ULS derivatized peptide (2^+ and 3^+ product ions).

Fig. 18



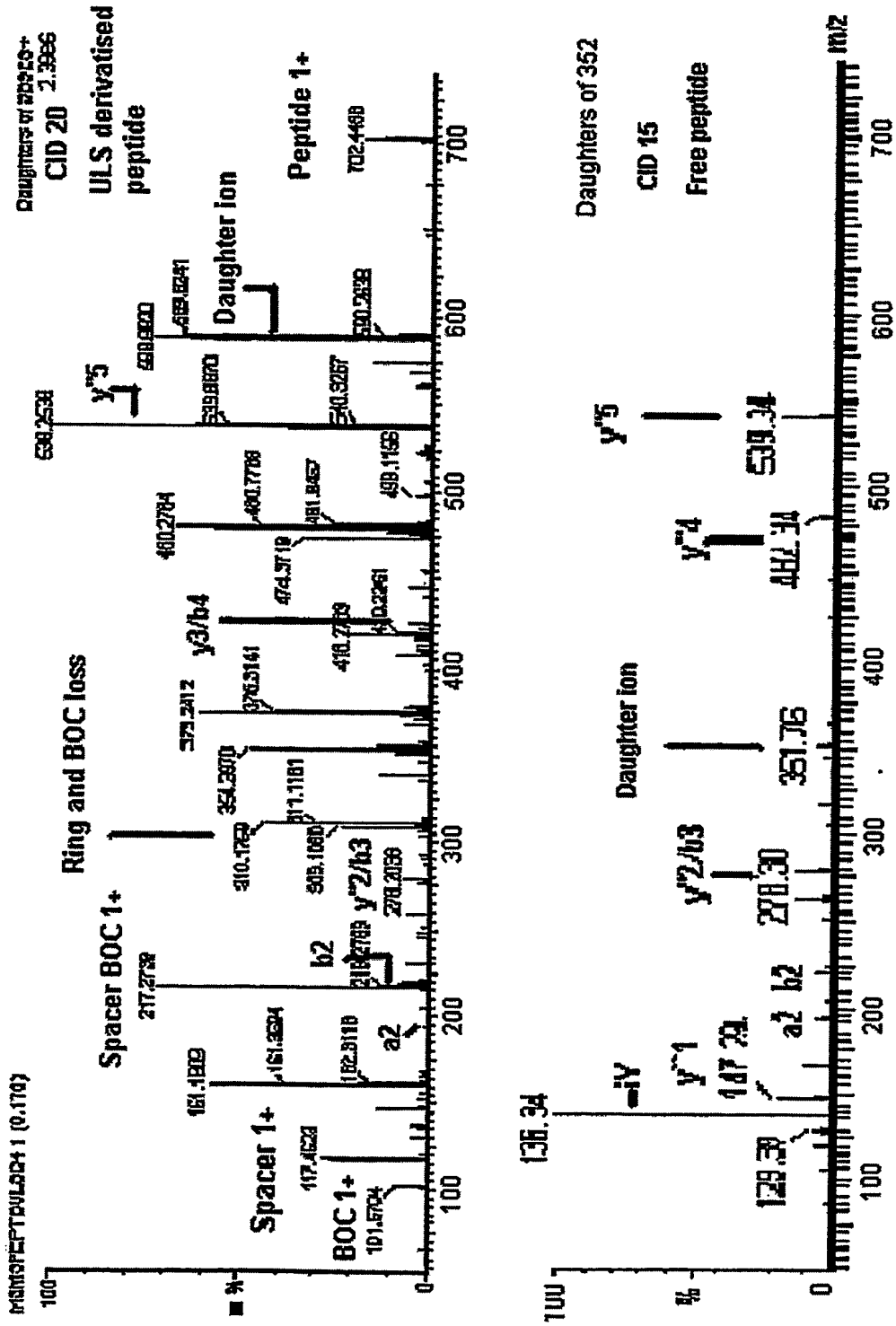
Direct infusion ESI of LWMR peptide, showing ionization efficiency of free peptide and ULS derivatized peptide (2^+ and 3^+ product ions).

Fig. 19



ESI MS/MS of YGGFMK. Lower frame shows tandem mass spectrum of YGGFMK using a CID energy of 15 eV. The middle frame shows the fragmentation pattern of the 3^+ product ion of D0 ULS [Cl/Boc] labelled YGGFMK using a CID energy of 8 eV and the top frame shows sequence ions of the 3^+ product ion of D0 ULS [Cl/Boc] labelled YGGFMK when the CID energy is increased to 15 eV.

Fig. 20



Lower frame shows tandem mass spectrum of YGGFMK using a CID energy of 15 eV.
The middle frame shows the sequence information obtained using the 2^+ product ion of D4 ULS [Cl/Boc] labeled YGGFMK for daughter ion scanning with a CID of 20 eV.